Mammalian Uroplakins

A GROUP OF HIGHLY CONSERVED UROTHELIAL DIFFERENTIATION-RELATED MEMBRANE PROTEINS*

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The asymmetric unit membrane (AUM) forms the apical plaques of mammalian urothelium and is believed to play a role in strengthening the urothelial apical surface thus preventing the cells from rupturing during bladder distention. We have shown previously that purified bovine AUMs contain four major integral membrane proteins: the uroplakins Iα (27 kDa), Iβ (28 kDa), II (15 kDa), and III (47 kDa). This contradicts some previous reports indicating that some of these proteins are absent in AUMs of several species. Using an improved procedure, we isolated AUMs from, in addition to cattle, eight mammalian species (human, monkey, sheep, pig, dog, rabbit, rat, and mouse). The AUMs of these species appear morphologically similar bearing crystalline patches of 12-nm protein particles with a center-to-center spacing of 16.5 nm. Using antibodies raised against synthetic oligopeptides or individual bovine uroplakins, we established by immunoblotting that the four uroplakins are present in AUMs of all these species. The DNA-deduced amino acid sequences of bovine and mouse uroplakin II revealed 83% identity. These results indicate that uroplakins Iα, Iβ, II, and III are the major protein components of probably all mammalian urothelial plaques, and that the sequence and three-dimensional structure of uroplakin molecules are highly conserved during mammalian evolution.

Ultrastructural studies have established that mammalian urothelium elaborate a highly specialized plasma membrane during an advanced stage of cellular differentiation. This membrane forms numerous rigid-looking plaques covering the apical surface of the urothelium. They exhibit a thickened luminal leaflet (8 nm) and a regular looking cytoplasmic leaflet (4 nm), hence the term asymmetric unit membrane (AUM)1 (Porter and Bonneville, 1963; Hicks, 1965; Koss, 1969). Negative staining of plaques purified from several species revealed densely packed, hexagonal protein particles (12 nm in diameter) that form crystalline patches associated with the outer leaflet of the AUM (Porter and Bonneville, 1963; Hicks, 1965; Hicks and Ketterer, 1969, 1970; Koss, 1969; Staehelin et al., 1972; Knutson and Robertson, 1976; Severs and Hicks, 1979; Robertson and Vergara, 1980; Wu et al., 1990). These plaques interact intimately with several important cell surface structures. Overlying these AUM plaques is a mucin layer which is believed to play a role in preventing bacterial adherence and colonization (Parsons et al., 1980; Pauli et al., 1983; Ruggieri et al., 1992). Underlying the plaques in the cortical cytoplasm is a meshwork of filaments, which appear to anchor the AUM to the cytoskeletal network. Such an AUM-cytoskeletal interaction is believed to be crucial in stabilizing the luminal surface thus preventing it from rupturing during bladder distention (Chlapowski et al., 1972; Staehelin et al., 1972; Minsky and Chlapowski, 1978; Sarikas and Chlapowski, 1986, 1989).

We have recently identified several major protein components of bovine AUM (Wu et al., 1990; Yu et al., 1990). Using a combination of sucrose gradient centrifugation and detergent treatment, we isolated from bovine bladder epithelium milligram quantities of highly purified AUMs. Upon SDS-PAGE analysis, these membrane preparations yielded three major proteins, a diffuse 47-kDa band, a broad 27-kDa band, and a 15-kDa band (Wu et al., 1990). Immunological studies using antibodies specific for each of these proteins have indicated that they are synthesized in terminally differentiated, superficial umbrella cells of bovine urothelium, and that their expression coincides with the appearance of AUM containing cytoplasmic vesicles and the luminal membrane (Wu et al., 1990; Yu et al., 1990). Immunoprecipitation and membrane solubility studies have revealed that these proteins form a stable complex, which remains insoluble not only in 9.5 M urea and 6 M guanidine hydrochloride but also in some nonionic detergents such as Nonidet P-40 (Wu et al., 1990). Immunoelectron microscopy has established that all these proteins are associated with the AUM in situ. Based on these data, we named these AUM subunits uroplakins, i.e. the 27-kDa uroplakin I, the 15-kDa uroplakin II, and the 47-kDa uroplakin III (Wu et al., 1990; Yu et al., 1990). Our recent molecular cloning data indicate that both uroplakin II and III possess a single membrane spanning domain. Their amino-terminal domains, which comprise the bulk of the molecules, are exposed to the luminal space (Wu and Sun, 1993; Lin et al., 1994). Further biochemical and molecular cloning studies on the 27-kDa uroplakin I has revealed the existence of two related proteins encoded by separate genes (Ryan et al., 1993; Yu et al., 1994). These two proteins show 39% sequence identity, and they are structurally similar in that both possess four transmembrane domains with two hydrophilic loops extending into the bladder lumen. We therefore designated these two proteins uroplakins Iα (27-kDa) and Iβ (28-kDa) (Yu et al., 1994).

Although ultrastructural studies suggest that AUMs are
characteristic of many mammalian urolithia, earlier biochemical studies of partially purified AUMs from several species yielded conflicting results. Using zonal centrifugation, Vergara et al. (1974) isolated pig AUMs which seemed to contain two major proteins (29 and 27 kDa). Caruthers and Bonneville (1977) identified only one major 33-kDa protein in a detergent-treated sheep AUM preparation. Stubbbs and co-workers (1979) described a 12- and a 22-kDa protein as the major constituents of bovine AUMs. Although these results suggest that interspecies variations exist in the protein subunits of AUMs, one cannot rule out the possibility that these apparent differences actually resulted from different isolation and analytical procedures. Whether uroplakins are conserved during mammalian evolution is therefore unclear.

In this paper, we describe a simplified procedure for isolating AUMs. Using this procedure, we have isolated AUMs from several additional mammalian species including human, monkey, sheep, pig, dog, rabbit, rat, and mouse. Digital image averaging of negatively stained crystalline AUM plaques revealed that the variously spaced antigen-containing protein particles can be resolved into 6 inner and 6 outer globular domains, with a highly conserved 16.5-nm lattice constant. Using rabbit polyclonal antibodies raised against intact bovine uropilaks as well as several synthetic oligopeptides corresponding to different epitopes of bovine uropilaks, we found that the four uropilak polypeptides, first described in bovine AUMs, are readily detectable in AUMs of all mammals tested. Uropilaks II and III were stained weakly, however, by Coomassie Blue and silver nitrate; this may explain why these two uropilaks were not readily detected by some of the earlier workers. A comparison of the cDNA- and genomic DNA-derived amino acid sequences of bovine and mouse UPIIs revealed an 83% sequence identity with a highly conserved prepro sequence containing a characteristic RGR cleavage site followed by the mature UPII polypeptide containing a COOH-terminal potential transmembrane domain. These results establish that the protein subunits of mammalian urothelial asymmetric unit membranes, i.e., the uropilaks, are highly conserved biochemically, immunologically, and structurally. Further studies on the three-dimensional structure, assembly, and gene regulation of these novel integral membrane proteins should yield new insights into urothelial function in health and disease.

MATERIALS AND METHODS

Isolation of Asymmetric Unit Membranes—Urinary bladders from various mammalian species were obtained 2–4 h postmortem. Asymmetric unit membranes were isolated using a procedure that we described previously (Wu et al., 1990) with, however, several modifications. Briefly, urothelial cells were scraped from the luminal surface of bladders, and washed three times with phosphate-buffered saline. The cells were homogenized in 10 mM HEPES/NaOH (pH 7.5) containing 1 mM phenylmethylsulfonyl fluoride, 1 mM EDTA, and 1 mM EGTA. After centrifugation at 2,000 × g at 4°C for 5 min, the pellet was resuspended in the same buffer, loaded onto a 1.6 × sucrose cushion, and centrifuged using an SW28 rotor (Beckman) at 16,000 rpm at 4°C for 20 min. The crude plasma membranes located at the interface were collected and washed with the above buffer by resuspension and centrifugation. These total plasma membranes were then treated with 2% sodium saccharide in 10 mM HEPES/NaOH (pH 7.4), 1 mM EDTA, 1 mM EGTA, and 1 mM phenylmethylsulfonyl fluoride at room temperature for 10 min. Finally, the detergent-resistant AUM were recovered by centrifugation at 18,000 × g at 4°C for 20 min, and washed with 10 mM HEPES/NaOH (pH 7.4). The AUM proteins were solubilized in 1% SDS and quantitated using the BCA reagent (Pierce Chemical Co.; Wu et al. (1990)).

Electron Microscopy and Digital Image Averaging—Purified AUM plaques were adsorbed to glow-discharged, carbon-coated collodion films mounted on copper grids (400 mesh per inch) and negatively stained with 0.7% sodium formates (pH 7.2). Images were recorded on Kodak SO-163 film at a nominal magnification of 50,000 under low-dose conditions using a Hitachi H-8000 transmission electron microscope operated at 100 kV. Micrograph areas yielding good optical diffraction patterns were digitized using an Eikonix 850 CCD imaging camera, 3 × 3 pixel averaged, thus sampling the specimen at ~0.64 nm. Using the SEMPER image processing system (Saxton et al., 1979), two methods were applied for averaging crystalline AUM patches: 1) quasi-optimal inner peak filtration (Aebi et al., 1973) to calculate the crystal quality and unit cell morphology, and 2) correlation averaging (Saxton and Baumeister, 1982) was used to eliminate intrinsic lattice disorder by locating the unit cells precisely using one hexagonal AUM unit cell as a reference. Finally, correlation-averaged unit cells were 6-fold symmetric (Saxton and Baumeister, 1982).

RESULTS

A Simplified Procedure for Isolating Asymmetric Unit Membranes—Taking advantage of the extreme insolubility of AUMs in a variety of detergents, we have previously purified bovine AUMs by a stepwise sucrose density gradient to first
enrich the AUM-containing membranes followed by removing the contaminating non-AUM membranes by dissolving them in 2% deoxycholate (Wu et al., 1990). This procedure took 2 days and yielded an average of 1 mg of highly purified AUM/bovine bladder. By directly isolating the total membrane fraction without first enriching AUMs by the discontinuous density gradient, and by using a stronger detergent (sodium sarcosine) to remove the contaminating non-AUM membranes, we have now shortened the procedure to 1 day with a comparable AUM yield and morphology (Figs. 1a and 2a; see below). As documented in Fig. 3a, lane 1, electrophoretic analysis of bovine AUMs purified this way revealed three major protein bands: a diffuse 47-kDa band, a broad band in the 27-kDa region, and a 15-kDa band. The morphological and biochemical features of bovine AUMs isolated this way were therefore in excellent agreement with those isolated previously (Wu et al., 1990; and data not shown).

**Structural Conservation of Mammalian AUMs**—Using this simplified procedure, we were able to purify AUMs from the urinary bladders of, in addition to cattle, eight mammalian species: human, monkey, sheep, pig, dog, rabbit, rat, and mouse. Examination of these AUMs by electron microscopy revealed that AUMs of these diverse mammals contained, very much like bovine AUMs, 12-nm protein particles forming hexagonally packed crystalline patches (Fig. 1, b-d). In order to compare the unit cell morphology of the AUM plaques among the different species in more detail, we performed digital image averaging. In all species that have been examined, the 6-fold symmetric unit cell (i.e. the "12-nm particles") consists of six stain-excluding regions surrounding a stain-filled central depression (Fig. 2, b-d). Each of these six elongated stain-excluding regions has a distinct, right-handed kink and can be resolved into an inner and an outer globular domain. The six inner domains with an average diameter of 3.3-nm define an inner annulus, centered at a radius of 3.7 nm, and the six outer domains with an average diameter of 2.7 nm define an outer annulus, centered at a radius of 6.6 nm. The six inner domains are rotated by about 12° counterclockwise, and the six outer domains by about 20° clockwise relative to the hexagonal lattice lines. In addition, there are two types of stain-excluding bridges, one connecting adjacent inner domains with each other, and the other connecting each inner domain with its closest outer domain (Fig. 2). These results confirm and extend the previous observations by Warren and Hicks (1970), Brisson
Fig. 2. Correlation-averaged and 6-fold symmetrized AUM unit cells of (a) cattle, (b) dog, (c) mouse, and (d) monkey. These averages were computed from electron micrograph data shown in Fig. 1. In all species the unit cells are arranged on a quasi-hexagonal lattice with lattice constants of \( a = b = 16.5 \) nm. The unit cells harbor 12-stain excluding domains which are arranged on two circles (\( r_{\text{in}} = 3.5 \) nm, \( r_{\text{out}} = 6.5 \) nm) each containing 6 domains. The outer domains are rotated by about 30° relative to the inner domains and the connections introduce a right-handed vorticity into the unit cell. Note the highly conserved AUM unit cell morphology in all four species shown. Scale bar, 5-nm (a-d).

Fig. 3. Resolution of uroplakins Ia and Ib, and the identification of a previously unrecognized, low molecular mass (15-18 kDa) component present in highly purified AUMs. AUM components from (a) cattle, (b) rabbit, and (c) mouse were dissolved in 1% SDS at room temperature, resolved by SDS-PAGE, and visualized by Coomassie Blue (lanes 1, 5 μg of total AUM proteins) or silver nitrate (lanes 2, 1 μg of total proteins) staining. The samples were not boiled because as we have shown earlier the 27-kDa UP1 undergoes heat-induced aggregation (Wu et al., 1990). Samples of parallel gels (with 1 μg of total AUM proteins) were electrotransferred onto nitrocellulose sheets and immunostained with a rabbit antibody against intact UPIII (lanes 3), or with antisera raised against various synthetic peptides corresponding to internal sequences of bovine UPIa (lanes 4), UPIb (lanes 5), and UP11 (lanes 7). Lanes 6 were stained with a mixture of anti-UPIa and anti-UPIb. The relative positions of bovine uroplakins I, II, and III are indicated on the left of panel a. Note that although UPIa and UPIb appear as a diffuse band in bovine AUMs, those of the rabbit and mouse AUMs are well-resolved. Also note in a and b that the UP11 region contains a protein band (arrows) which has a lower electrophoretic mobility than UP11. This band was barely visible in Coomassie Blue-stained gels, but became clearly visible after silver nitrate staining. It is immunologically distinct from UP11 since it is not recognized by anti-UP11 antibodies.

and Wade (1983), and Taylor and Robertson (1984), and indicate that the structural organization of mammalian AUMs is highly conserved.

Conservation of the Size and Immunogenicity of Mammalian Uroplakins—To determine whether the protein composition of mammalian AUMs is conserved, we resolved the AUM proteins of cow, rabbit, and mouse by SDS-PAGE, and stained them with Coomassie Blue (Fig. 3, lanes 1) or silver nitrate (Fig. 3, lanes 2). Individual uroplakins were identified by immunoblotting using antibodies that are monospecific for UPIII (lanes 3), UPIa (lanes 4), UPIb (lanes 5), and UPII (lanes 7). The results indicate that the protein profiles of rabbit (Fig. 3b) and mouse (Fig. 3c) AUMs are very similar to that of bovine AUMs (Fig. 3a), with protein subunits corresponding to all four bovine uroplakins.

As shown in Fig. 3c, UPIa and Ib of bovine were only partially resolved. However, UPIa and Ib of the rabbit and mouse, as detected by monospecific antibodies, are well separated (Fig. 3, b and c). In these cases, the lower and upper bands were identified as uroplakins Ia and Ib, respectively. Immunoblot-
As mentioned earlier, dient-purified bovine AUMs (containing both UPIa and UPIb after deglycosylation) were transferred to nitrocellulose paper and immunostained with antibodies to control uroplakins, while the heterogeneously transferred UPIb band became a single band as a result of deglycosylation. The resolved major 27-28-kDa components present in highly purified AUM preparations. Although the UPIb of cattle and mouse appeared to be heterogeneous (Fig. 3, a and c, lanes 5; also see Fig. 4, lane 4), deglycosylation of bovine UPIb resulted in the formation of a single, well-defined core protein (Fig. 4, lane 5), suggesting that the apparent heterogeneity reflects different degrees of glycosylation. As demonstrated earlier, so far the 15-kDa UP1I has been regarded to consist of a single protein species (Yu et al., 1990; Yu et al., 1990). The bovine protein band can sometimes be resolved, however, into two partially overlapping bands (Fig. 3a, lanes 1 and 2, arrow). The lower band appears to be more abundant according to Coomassie Blue staining, is not recognized by any of the anti-UPIII antibodies tested so far (lanes 7, and data not shown), and is recognized by four independent antisera that were raised against different synthetic peptides corresponding to different epitopes of the recently cloned bovine uroplakin II (see below). The upper band, on the other hand, is barely stained by Coomassie Blue (Fig. 3, a and b, lanes 1) but is stained strongly by AgNO₃ (Fig. 3, a and b, lanes 2), and is recognized by four independent antisera that were raised against different synthetic peptides corresponding to different epitopes of the recently cloned bovine uroplakin II (see below). The upper band is well resolved from UP1I (Fig. 3b, lane 2, arrow). Although such an upper band was not apparent in the AUMs of mouse and several other species (Figs. 3 and 5), we cannot rule out the possibility that in these species this band comigrates with UP1I. Since this band is present in significant amounts in highly purified bovine, sheep, and rabbit AUM preparations (Fig. 3, a and b; Fig. 5, lane 4), it most likely represents a genuine but hitherto unrecognized subunit of AUM. Finally, although the 47-kDa UP1II was barely detectable by Coomassie Blue and silver nitrate staining in the AUMs of rabbit and mouse (Figs. 3 and 5), it was readily detectable by immunoblotting (Figs. 3 and 6).

To determine more precisely whether the relative size and immunological properties of individual uroplakins are conserved, we compared on the same SDS gel the electrophoretic mobilities of the AUM proteins of nine mammalian species (Fig. 5). We also immunoblotted these proteins using a panel of antibodies, many of which were raised against synthetic oligopeptides corresponding to different regions of individual bovine uroplakins (Table I; Fig. 6). In addition to providing further evidence for the conservation of the overall polypeptide pattern of mammalian AUMs (Fig. 5), and for the immunological conservation of most of the uroplakin epitopes tested (Fig. 6), this analysis showed the following. First, we found that the AUMs of several species, when analyzed by SDS-PAGE without prior reduction, contain a 54-kDa protein (Fig. 6A). This protein is present in only a small amount in bovine AUMs (Fig. 4, lane 2), is recognized by antibodies to two different synthetic peptides of UPIa, and can be abolished by reduction (Yu et al., 1994). These results suggest that it most likely represents a homodimer of UPIa. These same AUM preparations do not, however, contain homodimers of UPIb (Fig. 6B). Second, as shown in Fig. 6, A and A', UP1b seems to be highly conserved with regard to its size (27 kDa), and two of its epitopes are completely conserved across all nine mammalian species. In contrast, as shown in Fig. 6, B and B', UP1b varies somewhat in its size (27-30 kDa) and antigenicity (both epitopes tested showed species variations). Interestingly, a polyclonal antiserum originally raised against total bovine AUM proteins was found to contain potent antibodies to UP1b (Fig. 6B'), but none to UPIa. This result indicates that UP1b is more antigenic than UPIa. Third, the size of UPII is quite conserved being detected as a 15-kDa component in most mammalian species. The UPII homologues of rat and mouse are, however, exceptionally large (17-18 kDa; Fig. 6, C and C'; see below). Fourth, the data provided further evidence that although the staining intensity of UPII and UP1II are in general quite weak and variable, they
FIG. 6. Immunological analyses of mammalian uroplakins using antibodies to synthetic bovine uroplakin peptides. AUM proteins (1 μg) of nine mammalian species were resolved by SDS-PAGE, electrophoretically transferred to nitrocellulose, and reacted with various rabbit antibodies raised against either intact uroplakins or synthetic peptides corresponding to specific cDNA-derived amino acid sequences of uroplakins (for the sequences of these peptides, see Table I). All these antisera are monospecific recognizing only one uroplakin (Wu et al., 1990; and data not shown). The anti-peptide-2 of bovine UPIa (UPIa); sera used here were raised against: (A) peptide-4 of UPIa; (B) peptide-1 of UPIb; (B') peptide-2 of UPIb; (B) intact UPIb protein (see text); (C) peptide-2 of UPII; (C') peptide-3 of UPI; and (D) intact UPIII. Numbers on the left denote relative molecular mass (MW) in kilodaltons (kDa). Abbreviations of species are as described in the legend to Fig. 5. Note that, with few exceptions, most of the epitopes of UPIa, II, and III are highly conserved, while the two epitopes of UPIb tested show significant species-to-species variations.

![Table I](image)

Antigenic specificity of the uroplakin antibodies

All of these are rabbit antibodies which by immunoblotting were shown to be monospecific for the corresponding uroplakin subunits. The amino acid sequences of UPIa and Ib, II, and III are according to Yu et al. (1994); Lin et al. (1994); and Wu and Sun (1995), respectively.

<table>
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<td></td>
<td></td>
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are both readily detectable by immunoblotting (Fig. 6, C, C', and D).

The Amino Acid Sequences of Bovine and Mouse UPII Are Highly Conserved—As a next step in examining more closely the species conservation of uroplakins, we compared the amino acid sequences of bovine and mouse uroplakin II. For this purpose, we used a bovine UPII cDNA (Lin et al., 1994) as a probe to isolate a mouse UPII genomic clone. Comparison of this mouse genomic sequence with the bovine UPII cDNA as well as the consensus sequences of exon-intron splicing junctions established that the mouse UPII gene is composed of four introns and five exons, the latter encoding a polypeptide of 184 amino acids (Fig. 7). As we have shown earlier, the polypeptide chain of bovine UPII is 185 amino acids long (Lin et al., 1994). The bovine and mouse UPII protein sequences were highly similar with 83% identity (boxed in Fig. 7) or 91% similarity, and shared several important features including the putative signal peptide cleavage site (Fig. 7, arrow), and the precursor processing motif "RGRR" (Fig. 7; Lin et al., 1994). Interestingly, the

polypeptide chain of mature mouse UP1 contains a potential N-linked glycosylation site which is absent in bovine UP1. This site, if indeed glycosylated in vivo, could explain the exceptionally high molecular mass of rodent UP1, i.e. 18 kDa in mouse versus 15 kDa in bovine and most other species (Fig. 5).

**DISCUSSION**

**Protein Composition of Mammalian Asymmetric Unit Membranes: How Many Uroplakins?**—We have shown previously that bovine AUMs contain at least four uroplakins molecules, designated UP1a, UP1b, UP11, and UP111 (Wu et al., 1990; Yu et al., 1990, 1994; Wu and Sun, 1993; Lin et al., 1994). In this study, we have shown that these four uroplakins are highly conserved and represent the major constituents of AUMs isolated from eight other mammalian species (Figs. 3, 5, and 6). The fact that an almost identical set of proteins has been repeatedly observed as the major components of all mammalian AUMs studied so far strongly suggests that these uroplakins represent true constituents of the AUM, some of them most likely involved in forming the 12-nm protein particles (Lin et al., 1994). The mature bovine UP11 consists of 13-15 kDa, a size similar to that of UP11 (Takahashi et al., 1990; Bradybury et al., 1992; Mitamura et al., 1992). Another important property shared by all members of this gene family is that they all have several highly conserved cysteine residues located in the major luminal loop connecting the 3rd and 4th transmembrane domains. We have recently shown that some of these cysteines are involved in forming intramolecular disulfide bonds possibly capable of stabilizing the conformation of this major luminal loop (Yu et al., 1994). Finally, the functional significance of having two closely related uroplakin I molecules is currently unclear. These two uroplakin I molecules differ in that UP11 is more conserved and can exist as a homodimer (Fig. 6A) (Yu et al., 1994). Co-purification data indicate that UP1b, and possibly UP1a, can bind to UP11 and UP111 (Yu et al., 1990). Future studies using bifunctional cross-linking reagents are needed to determine whether UP1 and UP1b can interact with each other.

**Uroplakin II (15 kDa)—**Our SDS-PAGE immunoblotting (Figs. 3, 5, and 6) and cDNA/gene sequence data (Fig. 7) have clearly established that UP11 is highly conserved during mammalian evolution, suggesting that it is functionally important. According to its cDNA-deduced protein sequence, and some actual protein data, we have recently shown that bovine UP11 is synthesized as a 28-kDa precursor with an N-terminal signal peptide followed by a heavily glycosylated prosequence, which may be important in regulating UP11's ability to interact with other uroplakins to form the highly insoluble uroplakin complex (Lin et al., 1994). The mature bovine UP11 consists of 100 largely hydrophilic amino acids which are predicted to form multiple β-sheets (Lin et al., 1994). The protein is anchored in the membrane through a COOH-terminal tail of hydrophobic, potential transmembrane domain. Preliminary chemical cross-linking data (not shown) indicate that bovine UP11 has a propensity to oligomerize, thus raising the interesting possibility that its β-sheets may be involved in forming a (luminal) β-barrel-like structure. Consistent with the fact that the electrophoretic mobility of the protein is not affected by deglycosylation, the mature bovine UP11 does not contain a potential N-glycosylation site based on its cDNA-deduced primary sequence (Lin et al., 1994). The mouse genomic DNA-derived data presented in Fig. 7 showed, however, that mouse UP11 contains a potential N-glycosylation site. Further experiments are needed to determine whether the glycosylation of such a site can account for the additional mass of rodent UP11 (Fig. 6).

The SDS-PAGE data shown in Fig. 3 established that highly purified rabbit AUMs contain a hitherto unknown 18-kDa protein, which is clearly distinct from other uroplakins since it is not recognized by multiple antibodies to uroplakins Ia, Ib, II,
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and III. Judging from the fact that this 18-kDa protein is present in significant amounts in highly purified AUMs, we strongly suspect that this protein is a genuine AUM subunit. We are currently testing this hypothesis by isolating and cloning this protein from rabbit AUMs taking advantage of the fact that in this species it is exceptionally well resolved from UP11 to possess a significant cytoplasmic domain. This raises the intriguing possibility that UP11 may play a role in the interactions of the AUM with components of the cytoskeleton (Wu and Sun, 1993). When AUM proteins of different species were detected by silver nitrate staining, the UP11 level seemed to vary significantly (Figs. 3 and 4). However, immunoblotting showed that roughly the same amounts of UP11 were detected in all the species examined except the monkey sample (see Fig. 6D). Whether some of the uroplakins of the monkey AUM preparation were degraded during isolation is unknown and needs to be further investigated.

Although the 47- to 49-kDa bovine UP11 appears as a diffuse band upon SDS-PAGE, we believe that this band consists of only uroplakin III because: 1) the amino acid composition of the electrophoretically purified 47-49-kDa protein matches precisely with that of cDNA-derived deduced values (Wu and Sun, 1993); 2) the cDNA-derived amino acid sequence of uroplakin III can account for all the partial peptides of the electrophoretically purified 47-49-kDa band (Wu and Sun, 1993); and 3) deglycosylation of the 47-kDa bovine glycoprotein yielded a single band of 30-kDa recognized by multiple antibodies against uroplakin III (Wu and Sun, 1993).

Correlation with Earlier Data—Given the highly conserved AUM structure consisting of crystalline patches of well resolved 12-nm protein particles packed as a 16.5-nm spacing hexagonal lattice (Figs. 1 and 2), one would expect a “fixed” and well defined subunit stoichiometry of uroplakins. However, SDS-PAGE analyses revealed a surprising degree of quantitative variation in the relative ratio of the various uroplakins (Fig. 5). These results are in agreement with those of Vergara et al. (1974) who showed that pig AUMs contained two major proteins of 27 and 29 kDa, which almost certainly correspond to our UP1a and Ib, respectively (see Fig. 5, lane 5). Our results are also in agreement with those of Caruthers and Bonneville (1977) who identified in sheep AUMs a single 33-kDa component which most likely represents a mixture of UP1a and Ib (Fig. 5, lane 4). The “22-kDa” and “12-kDa” proteins of bovine AUMs previously described by Stubbs et al. (1979) appear to correspond to UP1a and UP1II, respectively (Figs. 3a, 4, and 5, lanes 1). Therefore, the varied level of detectability of various uroplakins by Coomassie Blue and silver nitrate staining (Figs. 3 and 5) may explain why earlier investigators detected some but not other uroplakins. Such a variable staining reaction also makes it difficult at this present time to determine the precise stoichiometry of the uroplakins. The identification of at least four distinct AUM subunits challenges the previous assumption that the 12-nm protein particles represent oligomers of only one or two polypeptides (Vergara et al., 1974; Caruthers and Bonneville, 1977; Stubbs et al., 1979). The availability of a panel of antibodies recognizing specific epitopes of all major uroplakins (Table I; also see Wu and Sun (1993)) should enable us to correlate, at the ultrastructural level, these epitopes with different subdomains of the 12-nm protein particles.

Human Uroplakins—Although the AUM structure has been known to exist in many mammals including rat (Hicks, 1965), mouse (Walker, 1960), pig (Vergara et al., 1974), sheep (Caruthers and Bonneville, 1977), and cattle (Stubbs et al., 1975, 1979; Surya et al., 1990; Wu et al., 1990; Yu et al., 1990), its existence in human bladder epithelium has been controversial (Firth and Hicks, 1973; Pauli et al., 1983). Our results clearly documented, however, that the subunit composition of human AUMs is similar to that of other mammals (Figs. 5 and 6). Moreover, we have recently found that uroplakins are associated with the superficial umbrella cells of normal human bladder epithelium.5 These results firmly established the existence of uroplakins in human bladder epithelium. We are currently exploiting the possible use of the uroplakins as markers for diagnosing metastatic bladder neoplasms, and the possible involvement of uroplakin defects in certain bladder disorders such as interstitial cystitis.

Concluding Remarks—Taken together, the results presented in this paper clearly established that UP1a, UP1b, UP1II, and UP1III, that we first identified in bovine AUMs, are also present as the major protein subunits of AUMs of many other mammalian species, and that uroplakins are highly conserved regarding their relative size, antigenicity and, in the case of UP1II, in their amino acid sequences. Our data also indicate that these four uroplakin subunits form a highly conserved AUM structure. Such a high degree of structural conservation in the uroplakin molecules strongly suggests that they play an important functional role during an advanced stage of urothelial differentiation. Whether the AUMs indeed play a role in stabilizing the urothelial apical surface through AUM-cytoskeletal interactions needs to be determined experimentally. We are currently characterizing the 5'-promoter region of the mouse uroplakin genes using the transgenic mouse approach in the hope that we will later be able to produce a phenotype that would allow us to establish the specific in vivo function of the uroplakins.

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